pertussis toxin (PT), hemagglutinin (FHA), 69K membrane protein, or a partial peptide thereof, each of which is prepared by genetic engineering or chemical synthesis.

- Pertussis-diphtheria-tetanus combined vaccine: a trivalent vaccine prepared by mixing pertussis vaccine with diphtheria toxoid and tetanus toxoid, which is administered by injection, orally, percutaneously or intranasally.
- Japanese encephalitis vaccine: a vaccine containing antigen proteins that are obtained by (a) growing viruses in the mouse brain or in Vero cells using animal cell culture techniques, (b) purifying the virus particles by ultracentrifugation or with ethyl alcohol, and (c) inactivating with formalin; alternatively, antigen proteins can be obtained by genetic engineering or chemical synthesis.
- Hepatitis B vaccine: a plasma vaccine that is obtained using blood collected from hepatitis B carriers as raw material, and separating and purifying HBs antigen by salting-out, ultracentrifugation, and others; alternatively, a recombinant vaccine containing antigen portions obtained by gene engineering or chemical synthesis.
- Measles vaccine: a live vaccine of attenuated virus that is prepared by growing the viruses in culture cells such as chicken embryonic cells or in embryonated eggs; alternatively, a vaccine containing a part of the virus or a recombinant vaccine containing the protective antigen prepared by gene engineering or chemical synthesis.
- Rubella vaccine: a vaccine containing viruses grown in culture cells, such as chicken embryonic cells or in embryonated eggs, part of the virus or the protective antigen prepared by genetic engineering or chemical synthesis.
- Mumps vaccine: an attenuated live vaccine containing viruses grown in culture cells, such as rabbit cells or in embryonated eggs, part of the virus, or the protective antigen prepared by gene engineering or chemical synthesis.
- Measles-rubella-mumps vaccine; a trivalent vaccine that is obtained by combining measles vaccine, rubella vaccine, and mumps vaccine.
- Measles-rubella vaccine; a divalent vaccine that is obtained by combining measles vaccine and rubella vaccine.
- Rotavirus vaccine: a vaccine containing viruses grown in culture

cells such as MA104 cell, viruses collected from patient's feces, part of the virus, or protective antigen prepared by genetic engineering or chemical synthesis.

- *Mycoplasma* vaccine: a vaccine containing *Mycoplasma* grown in medium for *Mycoplasma*, a part thereof, or protective antigen prepared by genetic engineering or chemical synthesis.
- AIDS vaccine: a vaccine containing viruses grown in culture cells or viruses obtained from patients, part thereof, or protective antigen prepared by genetic engineering or chemical synthesis; alternatively, a DNA vaccine containing effective DNA fragments.
- *H. pylori* vaccine: a vaccine containing, as antigens, lysate of cultured *H. pylori*, or urease, heat shock protein, toxin and others separated from *H. pylori* culture; alternatively, a vaccine comprising these antigen proteins produced by genetic engineering, and administered by injection, oral inoculation, percutaneous inoculation or intranasal inoculation.

Properties of vaccine:

The vaccines above may be provided as liquid forms or powdered forms.

When each of these vaccines is administered together with an attenuated toxin adjuvant of the present invention, the liquid form of the vaccine is often more suitable for intranasal inoculation (intranasal spray, intranasal instillation, spread, etc.) and injection. Further, the intranasal inoculation can be performed with powder spray. The vaccine preparations of the invention can also be formulated with a conventional stabilizer or preservative. An illustrative stabilizer includes, but is not limited to, gelatin and dextran of about 0.2%, sodium glutamate of 0.1 to 1.0%, lactose of about 5%, and sorbitol about of 2%. Preservatives include about 0.01% thimerosal, phenoxyethanol of about 0.5%, and about 0.1% β-propiolactone.

Mixing ratio of attenuated toxin:

The mixing ratio between a vaccine antigen and an attenuated toxin adjuvant of the present invention can be, for example, 1:0.0001

to 1:10000 (by weight ratio) in vaccine preparations in accordance with the present invention. However, this range is merely illustrative and is not limiting on the vaccines of the present invention. A suitable ratio can be routinely selected depending on the type of vaccine. Methods required for selection are known to those skilled in the art. As seen in the Examples below, an inventive adjuvant comprising an attenuated toxin derived from a natural one in accordance with the present invention exhibits activity of enhancing immunity, the level of which is the same as that of the natural one when used at the same quantity. On the other hand, with a conventional recombinant mutant, it is often necessary to inoculate with a larger quantity to attain the same level of immuno-enhancing activity as that achieved with the natural toxin.

Mixing procedure of attenuated toxin:

The vaccine preparation of the present invention can be prepared by combining the above-mentioned vaccine with the attenuated toxin adjuvant of the invention at an adequate mixing ratio. The preparation must be done in a strictly sterile condition. Each of the raw materials must also be prepared under a completely sterile condition. It is desirable to remove contaminated proteins, i.e., those that have no vaccine activity and that act as pyrogens or allergens, as much as possible. Methods to achieve the treatment are conventional and known to those skilled in the art. The vaccine preparation of the invention can be effective even when the vaccine antigen and the attenuated toxin of the invention are separately prepared as pharmaceutical preparations and then the two are combined with each other at the time of inoculation or the two are inoculated at about the same time.

Vaccination method:

Any conventional method can be utilized for dosage pattern of the vaccine preparation in accordance with the present invention.

The dose is preferably about 5 to about 50 μl for intranasal inoculation to mouse; preferably about 0.1 to about 1.0 ml for the inoculation to human by intranasal administration or injection. Percutaneous inoculation can be performed by fixing the vaccine